

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : A61K 49/00, 33/04, 33/00 A61K 31/015, 31/02, A61B 8/14	A1	(11) International Publication Number: WO 93/06869 (43) International Publication Date: 15 April 1993 (15.04.93)
(21) International Application Number: PCT/US92/08385 (22) International Filing Date: 2 October 1992 (02.10.92) (30) Priority data: 07/771,300 4 October 1991 (04.10.91) US (71) Applicant: MALLINCKRODT MEDICAL, INC. [US/ US]; 675 McDonnell Boulevard, P.O. Box 5840, St. Louis, MO 63134 (US). (72) Inventor: VANDERIPE, Donald, R. ; 8 Auvergne Drive, Lake St. Louis, MO 63367 (US). (74) Agents: VACCA, Rita, D. et al.; Mallinckrodt Medical, Inc., 675 McDonnell Blvd., P.O. Box 5840, St. Louis, MO 63134 (US).	(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE). Published <i>With international search report.</i>	
(54) Title: GASEOUS ULTRASOUND CONTRAST AGENTS (57) Abstract <p>This invention relates to methods for ultrasound imaging utilizing a gas or mixture of gases capable of forming bubbles after administration.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	LI	Liechtenstein	SK	Slovak Republic
CI	Côte d'Ivoire	LK	Sri Lanka	SN	Senegal
CM	Cameroon	LU	Luxembourg	SU	Soviet Union
CS	Czechoslovakia	MC	Monaco	TD	Chad
CZ	Czech Republic	MG	Madagascar	TG	Togo
DE	Germany	ML	Mali	UA	Ukraine
DK	Denmark	MN	Mongolia	US	United States of America
ES	Spain			VN	Viet Nam
FI	Finland				

GASEOUS ULTRASOUND CONTRAST AGENTS

5 The present invention relates to compositions
A useful for ultrasound imaging and to the use of such
 compositions.

 Since the early days of the use of ultrasound for
 in vivo diagnostic imaging, there has been an ongoing
10 interest and effort to discover and develop agents
 suitable for providing contrast to the ultrasound
 images. Generally, gases have been found to provide
 the best contrast for intravascular images. The
 earliest success may have been the work of Gramiak et
15 al., Radiology 92, 939-948 (1969), wherein they
 demonstrated enhanced echogenicity following the rapid
 intravascular injection of various solutions in
 humans. Of those tested, the most echogenic was the
 dye indocyanine green which was observed to have
20 present in the bottle a minute amount of foam to which
 the contrast effect was attributed. Since then, many
 accounts of echogenic contrast have been reported for
 right heart studies, e.g. shunt detection, after i.v.
 injection of agitated solutions, including blood,
25 saline, X-ray contrast agent and the like, the
 agitation forming visible and smaller bubbles within
 the solutions. However, such agents injected
 intravenously are restricted to right heart imaging
 since the bubbles produced are too large to pass the

capillary bed of the lungs where the bubbles are trapped and easily broken. The use of such bubbles on the left side of the heart has been limited since the requirement of placing an arterial catheter is generally inconsistent with the noninvasive nature of the ultrasound examination. To provide for gas bubbles which can traverse the capillary bed of the lungs and provide for ultrasound echogenicity of the arterial circulation and organs subserved by the arterial circulation, most attempts have featured efforts to entrap or encapsulate gases within small size-controlled microspheres or particles composed, for example, of albumin, gelatin, sugars and the like. Such encapsulated gases may then be protected from trapping and breakage during passage through the pulmonary circulation. Although a number of early attempts were made to provide for this performance property, it was not until the mid-1980's that such agents were discovered as described in Feinstein U.S. 4,718,433.

Although these early attempts provide for contrast of the arterial circulation, they have certain disadvantages in their use. Such disadvantages include the need for some agents to be infused continuously or injected frequently in order to provide ongoing contrast because of the lability of these bubbles to the pressures encountered in the

arterial circulation. In these cases, relatively high doses of the encapsulating materials may need to be administered which may affect the safety of the agents. As well, the need to administer these agents by intravenous injections carries all the attendant disadvantages of accessing the vascular space, i.e. the needle puncture and risk of bleeding, clotting, hematoma, and disease transmission (hepatitis, etc.) Finally, none of these agents have a demonstrated ability to cross the capillary endothelium and enter the nonvascular extracellular fluid space or the intracellular space, therein limiting their usefulness to blood vessel contrast and not permitting tissue perfusion data in a manner similar to the well-established nuclear medicine procedure employing thallium-201 for heart imaging studies. Hence, the current agents offer a less than optimal method to provide for arterial ultrasound contrast.

An object of the present invention is to provide ultrasound agents which would obviate or lessen the problems and disadvantages cited above.

This invention relates to ultrasound contrast agents comprising pharmaceutically acceptable gases or gas mixtures capable of forming gas bubbles after administration in the arterial and/or arteriolar circulation and the tissues supplied by said circulation of a warm blooded animal. The gas bubbles

are of appropriate size to enhance ultrasound imaging. It was quite surprising, and contrary to teachings in physiology and pharmacology, that selected gases or gas mixtures after administration form gas bubbles within the arterial circulation under conditions which provide for diagnostic ultrasound contrast imaging in both 2D echo and Doppler contrast procedures. More importantly, these gases can be transported across the capillary and cell membranes and reform bubbles within the extracellular and intracellular space of the tissue to be imaged.

Another feature of this invention is the use of these agents to enhance the contrast of a diagnostic ultrasound procedure on a warm blooded animal. Still another feature is a sterile composition comprising the agent. Another feature is the use of said compositions to enhance the contrast of magnetic resonance imaging.

The gases and gas mixtures useful in the practice of this invention are best composed of gases which tend to form larger bubbles in the blood and tissues and might be typified by xenon and nitrous oxide and other weakly active general anesthetics such as sulfur hexafluoride. However, many other types of nontoxic gases, such as other perfluorocarbons, would be useful, provided they form bubbles in the blood of the appropriate size. Furthermore, the use of gas

mixtures under varying conditions of barometric pressure are useful and advantageous. For example, maximum blood/tissue contrast might be expected in cases where the inhaled partial pressure of the gas mixture is retained at a high percentage of the inhaled gases and the barometric pressure of the environment is reduced. In this situation, the size of the bubbles should grow and provide enhanced tissue contrast. Conversely, decreased contrast might be expected with smaller bubbles and, in cases wherein the subject is imaged under hyperbaric conditions, the latter compressing the gas back into solution. It may be possible to provide an assessment of relative differences in tissue perfusion merely through the inhalation of room air and imaging first under hypobaric conditions to optimize contrast and then reimage as the barometric pressure is increased to monitor the disappearance of contrast. Although such may be possible with room air, any differences should be enhanced by the inhalation of an optimized gas mixture.

The composition of such ultrasound contrast gas mixtures may be varied, but in practice should always contain oxygen to insure proper oxygenation if the gas is to be inhaled for a significant period of time. Typically, the mixture would be of two or three gases, but might, on occasion, include more complex mixtures.

The gas mixtures would be prepared according to the art using those available from the fractionation of air (oxygen, carbon dioxide, nitrogen, xenon, argon, krypton, neon, helium, etc.) and those prepared synthetically, such as the perfluorocarbons, etc. Normally, 15-25% of oxygen is present.

Although ultrasound contrast may be achievable using room air alone or under hypobaric conditions, optimized formulations and mixtures would be composed of gases which would form bubbles of optimum size in the tissues. Typically, these would be those gases which are poorly soluble in oil and somewhat more soluble in water and which would have a low partition coefficient when exposed to olive oil, i.e., those which would be described as weak or poor anesthetic agents (See Goodman & Gilman, The Pharmacologic Basis of Therapeutics, 8th Ed., 282). Such agents for maximum efficacy should have olive oil:gas partition coefficients of less than about 5:1 at 37 degrees centigrade, i.e., body temperature. Agents which would not generally be acceptable include the highly potent anesthetic methoxyflurane, and those less potent, but very effective, anesthetics such as chloroform, cyclopropane, halothane, enflurane, diethyl ether, fluoroxene and enflurane. Gases with maximum efficacy would include nitrous oxide, xenon, ethylene, sulfur hexafluoride, argon, and the like.

The basis on which the various gases might be optimized would be based on their oil:gas partition coefficients being reflected in the size of the gas bubbles which they form in the body formed within the body. Generally, the potent and clinically used anesthetics listed above form gas bubbles of 4 microns or less, most of which are less than two microns. Ultrasound is poorly reflected by such small bubbles and therefore these agents would be predicted to provide poor contrast especially at safe and/or subanesthetic concentrations. However, nitrous oxide can form gas bubbles in the body as large as 7-8 microns, a size which provides for excellent ultrasound reflectivity and those gases with similar oil:gas partition coefficients, namely, xenon and ethylene, would be expected to produce similar sized bubbles in the body (Goodman & Gilman above). As one progresses toward even lower oil:gas partition coefficients, e.g., sulfur hexafluoride and argon, the size of the bubbles should continue to increase, further enhancing the noted contrast. Although not clearly identified as to point of cut-off, it is probable that gases with very low oil:gas partition coefficients might become less effective by virtue of being too soluble in water. Nitrogen might be such a gas under most physiologic conditions and it has an oil:gas partition coefficient of about 0.1. Therein

the gases to provide in vivo ultrasound contrast when inhaled should optimally include those with olive oil:gas partition coefficients between about 10:1 (high) and about 0.01:1 (low), preferably from about 5:1 (high) to 0.1:1 (low). In addition, any gas mixture which needs to be inhaled for any significant length of time in order to provide optimal contrast, would need to have oxygen as a necessary component gas, usually at about 20% by volume.

Examples of effective gas mixtures would include:

- 1) 20% oxygen; 80% sulfur hexafluoride
- 2) 20% oxygen; 20% nitrogen; 60% nitrous oxide
- 3) 20% oxygen; 20% nitrogen; 60% xenon
- 4) 20% oxygen; 20% nitrous oxide; 60% sulfur hexafluoride
- 5) 20% oxygen; 20% xenon; 60% sulfur hexafluoride
- 6) 20% oxygen; 20% ethylene; 60% sulfur hexafluoride

Clearly, a number of the many synthetic fluorocarbon gases could be substituted for or added to sulfur hexafluoride.

In the practice of this invention, the agents are used in the usual manner. The gas or gas mixtures may be sterilized prior to administration or administered through a sterile filter. For example, an organ or tissue would be imaged prior to the administration of the gas mixture(s) in order to obtain a control tissue ultrasound signal level. This would be accomplished

using one of the readily available commercially marketed ultrasound machines such as Acuson, Toshiba, Hewlett Packard, ATL and the like. Then an agent of this invention would be administered by inhalation by the use of equipment well known to the art, either from a single gas container with the gases premixed or via the use of gas mixing valves and using two or more gas containers as appropriate. The inhaled gas mixture would then circulate to the tissues via the blood stream and be distributed to each tissue in a manner consistent with the blood supply to the tissue. By monitoring the ultrasound signal build up, either with 2D echo and/or color Doppler mapping, one could compare the blood flow to various tissues over time. Clearly, such procedures would be readily repeatable following short breathe-out periods and this would allow for both control and stress test studies to be conducted during a short time frame. The control (normal) test as described above would be repeated after exercise or following the administration of pharmacologic stress agents to assess blood flow deficits or slower signal build up in low flow areas of the body. Such rest and stress protocols have been used for some time in nuclear medicine as the thallium (Tl-201) stress test.

Typically, the inhalation of gases to provide the above ultrasound contrast will be for times suitable

for ultrasound imaging, usually short periods, generally less than about five minutes, but could be prolonged depending on the clinical application, e.g., monitoring of the return of tissue perfusion after a period of ischemia. Conversely, one might inhale the gas mixture for a very short period of time in order to get transient type information such as first pass organ/tissue perfusion, etc. Therein it is envisioned that the gas or gas mixtures might be inhaled over broad time frames with the clinical use determining the individual administration protocol.

It is to be understood that the invention is not to be limited to the exact details of operation or exact compositions or procedures shown or described. Obvious modifications and equivalents will be apparent to one skilled in the art, and the invention is therefore to be limited only by the scope of the appended claims.

Claims

1. The method of ultrasound imaging of a warm blooded animal comprising administering an ultrasound contrast agent comprising a pharmaceutically acceptable gas or mixture of gases in an ultrasound diagnostic amount for a period suitable to image and thereafter ultrasound imaging said animal, said gas or mixture of gases being capable of forming bubbles after administration of a size appropriate to enhance imaging in the arterial or arteriolar circulation and tissues supplied by said blood circulation.

2. A composition for ultrasound imaging comprising a pharmaceutically acceptable gas or mixture of gases, said gas or mixture of gases being capable after administration of forming bubbles of a size appropriate to enhance imaging in the arterial or arteriolar circulation and tissues supplied by said blood circulation.

3. The composition of claim 2 wherein the ultrasound said gas is xenon.

4. The composition of claim 2 wherein said gas is ethylene.

5. The composition of claim 2 wherein said gas is nitrous oxide.

6. The composition of claim 2 wherein said gas is sulfur hexafluoride.

7. The composition of claim 2 wherein said gas is argon.

8. The composition of claim 2 wherein said gas is selected from the group consisting of a perfluorocarbon gas.

9. The composition of claim 5 wherein the agent additionally contains oxygen.

10. The composition of claim 6 which additionally contains oxygen.

11. The method of claim 1 wherein said gas is xenon or a mixture of xenon and oxygen.

12. The method of claim 1 wherein said gas is ethylene or a mixture of ethylene and oxygen.

13. The method of claim 1 wherein said gas is nitrous oxide or a mixture of nitrous oxide and oxygen.

14. The method of claim 1 wherein said gas is sulfur hexafluoride or a mixture of sulfur hexafluoride or oxygen.

15. The method of claim 1 wherein said gas is argon or a mixture of argon and oxygen.

16. The method of claim 1 wherein said gas is selected from the group consisting of perfluorocarbon gas and mixtures.

17. The method of claim 1 wherein the ultrasound contrast gas procedure is used to image cardiac perfusion at rest and following exercise.

18. The method of claim 1 wherein the ultrasound procedure utilizes the modes of 2D echo, doppler, color doppler or color mapping.

19. The method of claim 1 wherein the ultrasound procedure is used to assess the perfusion of any peripheral noncardiac organ or tissue both at rest and after exercise.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/08385**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) :A61K 48/00, 33/04; 33/00, 31/015, 31/02; A61B 8/14

US CL :424/9; 424/600; 514/743; 514/759; 514/757; 128/662.01; 128/662.02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/9; 424/600; 514/743; 514/759; 514/757; 128/662.01; 128/662.02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	US, A, 5,088,499 (Unger) 18 February 1992, See column 2.	1-19
Y	US, A, 4,657,756 (Razor et al.) 14 April 1987, See column 3.	1-19
Y	US, A, 4,681,119 (Razor et al.) 21 July 1982, See abstract.	1-19
Y	US, A, 4,718,433 (Feinstein) 02 January 1988, See column 1 and 2.	1-19
P,Y	US, A, 5,078,146 (Sato) 07 January 1992, See abstract.	1-19

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be part of particular relevance

E earlier document published on or after the international filing date

L document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Z document member of the same patent family

Date of the actual completion of the international search

06 NOVEMBER 1992

Date of mailing of the international search report

21 DEC 1992

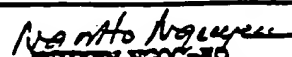
Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. NOT APPLICABLE

Authorized officer

For MARK W. RUSSELL

Telephone No. (703) 308-1235


 ROYEN 8300-80
 INTERNATIONAL DIVISION

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/08385

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS

ultrasound, bubble, xenon, argon, perfluorocarbon, oxygen, ethylene, nitrous oxide, arteriolar, arterial, 2D echo, color doppler, color mapping